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ET-170 December 1940

United States Department of Agriculture
Bureau of Entomology and Plant Quarantine

TECHNIQUE EMPLOYED IN PRODUCING UNIFORM PEA APHID STOCK FOR INSECTICIDE TESTS AT MADISON, WIS.

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Introduction

The first greenhouse experiments using rotenone-bearing dust mixtures and sprays against the pea aphid (Macrosiphum (Illinoia) pisi (Kalt.)) were staged by utilizing pea plants infested with a mixed population of the aphid. The proportion of different stages to the whole population differed greatly, not only from one test to another but frequently on different replicates of the same test. The results of some of these tests were erratic because rotenone-bearing dusts and sprays are much more quickly toxic to the early stages of the pea aphid than to the later stages.

It was decided that, if significant results were to be obtained, especially under conditions in which the probable range in mortality would amount to only 10 or 15 percent, populations uniform both as to stage and age must be employed. Therefore the technique described herein was devised, which has made it possible to obtain satisfactory uniformity in both pea plants and aphids.

Stock Plants

Pea plants upon which aphids are produced, for subsequent transfer to test plants, are grown in 6-inch flowerpots. A mixture of 3 parts of compost to 1 part of sand constitutes a satisfactory soil for their growth. Pots are filled about three-fourths full of the mixture and topped with 1 inch of sand. Root rot is largely controlled by dusting the seed, before planting, with a commercial mercuric compound sold under the name of Semesan, Jr. Wilt-resistant Perfection pea seed is then planted 1 inch deep in the sand, 9 or 10 to a pot, the seeds being uniformly distributed but away from the periphery of the pot, so as to allow room later for a screen cage. After planting, the soil is watered thoroughly and the pots are covered with cardboard until the seed has sprouted. The growing plants are watered thoroughly about twice a week. They respond

much better to occasional thorough watering than to frequent light watering. To rear approximately 1,500 adult aphids per week, 15 six-inch pots containing 7 or 8 plants each are required.

When stock plants are 14 days old they are of sufficient size to be infested. They should not be allowed to become overcrowded with aphids, for overcrowding induces dispersion and stimulates the production of alate forms. Therefore no more than 25 apterous adult aphids, which already have begun to reproduce, are placed on the plants in each pot, caged, and allowed to reproduce for 24 hours. At the end of this period the cages and all the adults are removed, disturbing the nymphs as little as possible. This procedure limits the age range of the ensuing adults to 24 hours. The time required for new-born nymphs to develop into adults varies with the temperature and the condition of the plants, but under an average greenhouse temperature of 70° F. and artificial light about 7 days are necessary.

Previously the practice was followed of placing larger numbers of reproducing adults upon fewer plants and subsequently transferring the second- or third-stage nymphs to fresh uninfested plants. It was found, however, that after the transfer many of the nymphs remained on the under side of the lower leaves and developed more slowly than did those which reached the upper leaves. By allowing nymphs to remain undisturbed on the plants on which they are born, a more uniform aphid stock is obtained.

Test Plants

Test plants are planted at the proper time (see table 2) so that they will be large enough to be infested when the aphids have reached maturity. Two test plants 14 days old will support 35 aphids and their progeny for 3 or 4 days if the temperature during a test is not too high.

Test plants are grown easily in ordinary sand, watered, and nourished with a complete nutrient solution. Number 2 enameled tin cans have been found satisfactory for growing test plants. There are several advantages in the use of tin cans over flowerpots. The cans are much cleaner and easier to handle. They take up less room in a temperature-humidity control chamber than do flowerpots of the same capacity. As the period during which each lot of plants is grown does not exceed 3 weeks, no difficulty has been experienced in obtaining natural aeration of the roots. Therefore it is not necessary to provide drainage openings in the can and thereby lose most of the nutrient solution supplied the plants. Lastly, the cans eliminate the escape or absorption of moisture through their sides and bottoms and allow the relative humidity in the control chamber to be more easily regulated.

The cans are filled with dry pit-run sand to within three-fourths of an inch of the top. It is important to start with dry sand for filling the cans in order that the quantity of water first supplied and the nutrient solution subsequently supplied will be favorable for germination and growth of the peas. Seed, treated the same as for stock plants, is planted three-fourths of an inch deep with two seeds to the can, and about 150 cc. of water are added. Nutrient solution in the amount of 50 cc. is supplied when the young peas unfold their first leaves (fig. 1), and the same quantity is supplied every 3 days thereafter, the last lot being added just before the plants are prepared for infesting (see table 1).

To eliminate the necessity of adding nutrient solution to the plants during a test, and also to prevent excessive escape of moisture into the temperature-humidity control chamber, the sand in each can is covered with a waxed-paper disc just before the test aphids are transferred. The can is then nearly filled with white quartz sand (fig. 2), which not only aids in moisture retention but is of considerable help in providing a light background upon which dead aphids may be easily seen and counted. White sand is not employed for growing the peas because that available in Madison, Wis., appears to contain some chemical slightly toxic to the plants.

It is not advisable to reuse sand after nutrient solution has been employed, as there is an accumulation of certain nutrient salts which may result in an unbalanced solution for the following lot of plants.

In order to provide fairly uniform test plants, it has been found desirable to plant about 50 percent more cans than will be needed for a test, although the excess number necessarily will vary according to the conditions, particularly the germination of the seed.

After being infested, the test plants are covered with cages made of 20-mesh screen wire to confine the aphids. A convenient size of cage is one 8 inches high and of a circumference to fit snugly inside the can. The more snugly a cage fits in the can, the less likely it is to fall off in handling.

Method of Infesting

Approximately 7 days after the stock plants are first infested with adult aphids, the nymphs produced by them should be ready to transform to adults. For the sake of both accuracy and speed in making the transfer of a large number of aphids, their transfer from stock plants to test plants should not be commenced until the large majority of nymphs have become adults.

The adult stage of the aphid was chosen for greenhouse testing of rotenone-bearing insecticides in order to eliminate any variation in the results which might be caused by the molting of the immature stages and also to obtain a uniform population consisting of only one stage. Nymphs produced during a test are ignored in recording the results.

Aphids are best removed from stock plants by laying a pot on its side with the plants over a piece of black painted tin, such as a photographic ferrotype plate, and then breathing heavily on them. The adult aphids will quickly drop from the plants. Adults should never be brushed or knocked off plants because of the danger of breaking the proboscis, which, of course, precludes their use as test aphids.

For counting aphids and placing them on test plants it has been found convenient to make use of a 100-cc. Erlenmeyer flask and a 2½-inch glass funnel. The adult aphids on the ferrotype plate are brushed with a camel's-hair brush into the mouth of the funnel inserted in the flask. The required numbers of aphids are then transferred from the flask to the plants and caged. Several hours elapse before treatment is begun in order to allow the aphids to establish themselves on the new plants. The screen cages are removed during the application of an insecticide.

Artificial Lighting

During winter months, when there is a deficiency in sunlight, it has been found absolutely necessary to provide artificial light for stock plants and test plants in order to produce both healthy plants and aphids. From 2 to 3 days also is saved in the time required to grow stock and test plants to the proper size for infestation. For this purpose 500-watt lamps in 16-inch porcelain reflectors are suspended about 2 feet above the plants. One lamp will adequately take care of 25 to 30 pots of plants or about 60 cans of plants.

Nutrient Solution for Peas

The nutrient solution is adapted from the one used by the Department of Plant Pathology of the University of Wisconsin. Distilled water should be used for mixing the stock solutions, but tap water usually is satisfactory for making the final solution. It is not necessary to use chemically pure materials. A technical grade of salts may be used. Stock solutions may be kept for a considerable period of time, probably a year at least, but the complete diluted solution should be used within a few days after being prepared. A Mohr pipette of 1 or 2 cubic centimeters' capacity, graduated in tenths of a cubic centimeter, is suitable for measuring the small quantities of minor salt solutions. The formula for preparing this nutrient solution is presented in table 1.

Table 1.--Formula for preparing complete nutrient solution for peas. Madison, Wis., February 1940

Major salts	Molecular weight in grams	Grams, per liter of distilled water, to make stock solution	Cubic centimeters of stock solution to add in making 15 liters of complete solution
Calcium nitrate ($Ca(NO_3)_2.4H_2O$)	236.15	118.08	90
Potassium nitrate (KNO_3)	101.10	50.55	90
Magnesium sulfate ($MgSO_4.7H_2O$)	246.50	123.25	73
Potassium phosphate (KH_2PO_4)	136.14	68.07	18
Sodium chloride (NaCl)	58.45	29.23	10
Minor salts			
Boric acid (H_3BO_3)	61.84	28.1	0.7
Cupric chloride ($CuCl_2.2H_2O$)	170.52	0.4	0.7
Zinc chloride ($ZnCl_2$)	136.29	0.3	0.7
Manganese chloride ($MnCl_2.4H_2O$)	197.91	3.88	0.7
Ferric chloride ($FeCl_3.6H_2O$)	270.31	5	27

It is advisable to add the solutions of the various salts to a carboy already filled with approximately 15 liters of water so that they will not be brought together in too concentrated a form.

The ferric chloride solution should be added last. Never add ferric chloride to concentrated solutions of the other salts, because precipitation will result.

Planting and Infesting Schedule

As an aid in carrying out the different operations required in producing aphids for tests, a planting and infesting schedule was prepared and is closely followed, because a delay in executing some of the preliminary operations may interfere with the proper timing of the experiment later on. This schedule is based upon experiments with the Perfection variety of peas. The schedule is presented in table 2.

TABLE 2.

SAMPLE SCHEDULE FOR PLANTING AND INFESTING STOCK PEA PLANTS AND TEST PEA PLANTS UNDER GREENHOUSE CONDITIONS.
Average Temperature 70°F. Plants and aphids under artificial light during the night and on cloudy days.
Predicated upon one test per week.
Madison, Wisconsin, February, 1940.

Days→	1st	6th	9th	14th	15th	16th	17th	20th	22nd	23rd	24th	25th	28th	30th	31st	
Stock plants	Plant in pots and cover	Plants up. Uncover			Infest plants with 25 adults per pot	Remove and discard all adults	----Nymphs developing----			Transfer* adults to test plants. Discard plants						
Test plants			Plant in cans	Add 50 cc. nutrient sol.			Add 50 cc. nutrient sol.	Add 50 cc. nutrient sol.		Add 50 c.c. nutrient solution. Infest plants	-----Test-----					
Stock plants			Plant in pots and cover	Plants up. Uncover						Infest plants with 25 adults per pot	Remove and discard all adults	---Nymphs developing---			Transfer* adults to test plants. Discard plants	
Test plants							Plant in cans		Add 50 cc. nutrient sol.			Add 50 cc. nutrient sol.	Add 50 cc. nutrient sol.		Add 50 cc. nutrient solution. Infest plants	--Test-
Stock plants							Plant in pots and cover		Plants up. Uncover						Infest plants with 25 adults per pot	etc.
Test plants												Plant in cans		Add 50 cc. nutrient sol.		etc.

*About 375 adults should be saved for infesting the new lot of stock plants.

Figure 1.--Supplying nutrient solution to peas grown in tin cans.

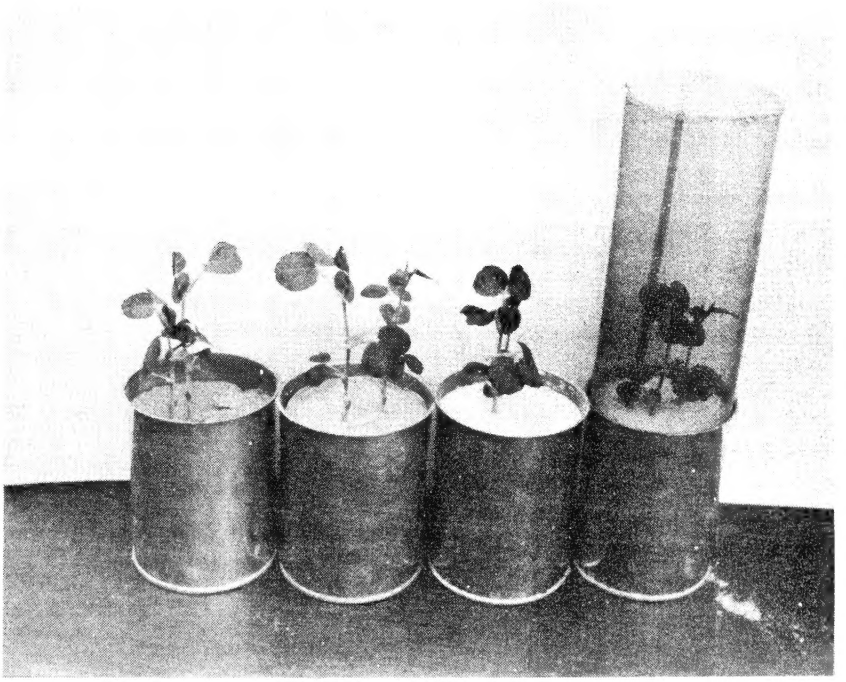

Figure 2.--Steps in preparing test plants for infestation.
Left--Fourteen-day-old plants growing in sand.
Next--Waxed-paper disk placed on top of sand to retain moisture.
Next--Waxed paper covered with white quartz sand.
Right--Prepared and infested plants covered with 16-mesh screen cage.